

Preclinical Considerations:

Toxicology studies are a major part of the preclinical safety evaluation in the drug development. These studies characterize the toxicity profile of a drug. The characterization identifies whether the drug damages any organ structurally or functionally in animals. It determines the severity and reversibility of the toxicity. It also determines a dose level at which no adverse effect will be observed.

Another integral part of preclinical safety evaluation investigates the relevance of drug toxicities in animals to humans. Toxicological findings often occur in some animal species, but not in others. An animal species exhibiting similar drug profile (pharmacology and pharmacokinetics) to that of humans will be considered more relevant to humans. Toxicological findings in these relevant species will be given more consideration. When there is a lack of evidence of human relevancy among animal species, toxicological findings in the most sensitive species will then be given the most consideration.

The preclinical safety evaluation also determines the margin of safety of a drug. The margin of safety is defined as the difference (multiples) in doses between the no-adverse-effect-level (NOAEL) in animals and the expected clinical dose in humans. The safety margin can be determined on the basis of weight (mg/kg/day), body surface area (mg/m²/day), or the plasma drug level defined as the area under the curve (AUC). In the Ariflo case, the AUC is used to determine the margin of safety. A small safety margin suggests that the drug may more likely cause similar damages in humans at the recommended clinical doses. The margin of safety derived from the most sensitive species is of more value if the relevant animal species can't be identified. This is the case for Ariflo.

Toxicological studies of Ariflo in laboratory animals show that the drug causes a variety of lesions to several organs after repeat-dose treatment. Mice, rats, rabbits, and monkeys were treated with Ariflo for up to 24, 24, 0.5 and 12 months, respectively. Dogs were not included in the drug development because of severe emesis after single administration. The drug causes damage to artery, adrenal cortex, testes, heart, gastrointestinal tract and the mammary glands in animals. The lesions include vasculitis in multiple organs, testicular degeneration of seminiferous epithelium, adrenal cortex hypertrophy and hyperplasia, myocardial necrosis, gastrointestinal disturbance and mammary gland tumors in animals. Some lesions occur in animals at an AUC that was only a small fraction of the expected human AUC in humans. This indicates there is no margin of safety between the NOAEL in animal and the expected clinical dose in humans.

The following section briefly describes each of the above lesions.

A. Vasculitis

Vasculitis, the inflammation of the blood vessels, occurred in rats and mice, but not in monkeys. Vasculitis was observed in multiple organs in rats and mice at oral doses of 30 and 200 mg/kg/day, respectively. The vascular lesion is characterized by medial necrosis in the arteries frequently accompanied by perivascular fibrosis and inflammatory cells. Arteritis was observed in the small vessels of the liver, stomach, mesentery, pancreas, intestines, adrenals, epididymis, testes and thymus. Severe vasculitis resulted in deaths within a few days treatment. The lesion is irreversible. The NOAEL is 20 and 100 mg/kg/day in the rat and mice, respectively. Vasculitis was not observed in the monkey at doses up to 10 mg/kg. Rats are the most sensitive animal species. Based on the AUC of the NOAEL in rats, there is no safety margin for vasculitis (Table 1).

Table 1. Safety Margin for Vasculitis

Species	NOAEL		Safety ^a
	mg/kg/day	AUC (mcg.h/ml)	Margin
Rat	20	4	0.2
Mouse	100	157	7
Monkey	10 ^c	88	4
Human		22 ^c	-

- a. Derived by dividing AUC at NOAEL in animals by the expected human AUC of 22 mcg.h/ml.
- b. Highest tested dose.
- c. At the maximum recommended dose of 30 mg/day.

B. Testicular Degeneration

Testicular degeneration was observed in rats and rabbits, but not in monkeys. Rats and rabbits treated with respective oral doses of 40 and 60 mg/kg/day showed degeneration of the testicular seminiferous epithelium. The degeneration was characterized by minimal to mild change of the tubular epithelium that lacked spermatozoa and contained multinucleated giant cells. The NOAEL was 20 and 30 mg/kg/day in rats and rabbits, respectively and the effect was not observed in the monkey at doses up to 10 mg/kg (Table 2).

There is a significant margin of safety between rabbits and humans. However, the rabbit were treated for only 2 weeks and such a short treatment duration is not considered adequate to assess the safety of chronic clinical use. Therefore, the safety margin should be based on the NOAEL in the rat, the most sensitive species. There is no safety margin.

Table 2. Safety Margins for Testicular Toxicity

Species	NOAEL		Safety ^a
	mg/kg/day	AUC (mcg.h/ml)	Margin

Rat	20	4	0.2
Rabbit	30	100	5
Monkey	10 ^b	88	4
Human		22 ^c	-

- Derived by dividing AUC at NOAEL in animals by the expected human AUC of 22 mcg.h/ml.
- Highest tested dose.
- At the maximum recommended dose of 30 mg/day.

C. Adrenal Cortex

Effect of Ariflo on adrenal cortex was evaluated in rats and monkeys. Ariflo induced changes in the adrenal cortex at oral doses of 5 mg/kg and higher in the rat, but not in the monkeys at doses up to 10 mg/kg (Table 3). The changes in the rat were cortical hypertrophy. The cortical hypertrophy was characterized by thickening of the inner cortical zones (zona fasciculate and reticularis). Cells of the zona reticularis were larger and more eosinophilic than normal making the zona more difficult to distinguish from the zone fasciculate. The rat is the most sensitive species. There is no NOAEL or margin of safety for these findings in the rat.

Table 3. Safety Margins for Adrenal Toxicity

Species	NOAEL		Safety ^a
	mg/kg/day	AUC (mcg.h/ml)	Margin
Rat	< 5	< 3	none
Monkey	10 ^b	88	4
Human		22 ^c	-

- Derived by dividing AUC at NOAEL in animals by the expected human AUC of 22 mcg.h/ml.
- Highest tested dose.
- At the maximum recommended dose of 30 mg/day.

D. Myocardial necrosis

Ariflo induced focal myocardial necrosis in rats and mice at respective oral doses of 80 and 400 mg/kg/day. The NOAEL was 40 and 100 mg/kg/day in rats and mice, respectively (Table 4). Monkeys at Ariflo doses up to 10 mg/kg did not show cardiac toxicity. There is no margin of safety based on the rat data.

Table 4. Safety Margins for Cardiac Toxicity

Species	NOAEL		Safety ^a
	mg/kg/day	AUC (mcg.h/ml)	Margin
Rat	40	12	0.5

Mouse	100	157	7
Monkey	10 ^b	88	4
Human		22 ^c	-

- Derived by dividing AUC at NOAEL in animals by the expected human AUC of 22 mcg.h/ml.
- Highest tested dose.
- At the maximum recommended dose of 30 mg/day.

E. GI disturbance

Ariflo causes gastrointestinal disturbances in all animal species tested. The disturbances are characterized as emesis and irritation. Emesis was observed in the monkey at doses of 1 mg/kg and higher. Erosion of mucosa was found in rats and mice at oral doses of 40 and 30 mg/kg/day, respectively. The NOAEL for erosion was 20 and 10 mg/kg/day in rats and mice, respectively. There is no margin of safety for emesis/erosion in rats, mice, or monkeys (Table 5).

Table 5. Safety Margins for Gastrointestinal Toxicity

Species	NOAEL		Safety ^a
	mg/kg/day	AUC (mcg.h/ml)	Margin
Rat	20	4	0.2
Mouse	10	8	0.4
Monkey	1	11	0.5
Human		22 ^b	-

- Derived by dividing AUC at NOAEL in animals by the expected human AUC of 22 mcg.h/ml.
- At the maximum recommended dose of 30 mg/day.

Summary:

Preclinical studies of Ariflo in laboratory animals have shown that the drug causes the inflammation of blood vessels in mice and rats, degeneration of testicular epithelium in rats and rabbits, hyperplasia and hypertrophy of adrenal cortex in rats and monkeys, myocardial necrosis in mice and rats and GI disturbances in mice, rats, rabbits and monkeys. AUCs at NOAEL in animals were smaller than that obtained from the clinical dose in humans. Thus, there is no sufficient safety margin to support the safety of the proposed clinical dose.